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Headspace volatile organic compounds as indicators of *Fusarium* basal plate rot and *Penicillium* rot in stored onion bulbs

Isabella Kleman [a,*], Anna Karin Rosberg [a], Oleksiy Guzhva [a], Maria E. Karlsson [a], Paul G. Becher [b], Lars Mogren [a]

[a] Department of Biosystems and Technology, Swedish University of Agricultural Sciences, PO Box 190, SE-234 22, Alnarp, Sweden
[b] Department of Plant Protection Biology, Swedish University of Agricultural Sciences, PO Box 190, SE-234 22, Alnarp, Sweden

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ABSTRACT

Food loss is a global problem, and one important contributor is the pathogen-induced loss of crops during storage in the supply chain. This could be countered by monitoring systems, including ones that monitor the release of volatile organic compounds (VOCs) indicating pathogen infection. In order to study the release of VOCs from onion bulbs affected by *Fusarium* basal plate rot or *Penicillium* rot bulbs were infected with the causal agents *Fusarium oxysporum*, or *Penicillium polonicum* and emitted headspace VOCs were analysed using solid phase microextraction (SPME) and gas chromatography coupled with mass spectrometry (GC-MS). We identified headspace VOCs that mark onion bulbs with pathogen infection. Seven compounds were detected in *F. oxysporum*-infected but not in healthy bulbs: 1-(methylthio)-propane, dimethyl disulfide, ethenylbenzene, 2,2-bis(methylthio)-propane, 1-(methylsulfinyl)-propane and 2-(methylsulfonyl)-propane and methyl 1-propenyl disulfide. In the volatile profiles of *P. polonicum*-infected bulbs a few compounds, including 2,4-dimethyl-thiophene and 2-hexyl-5-methyl-3(2H)-furanone were found in increased abundance. Accelerated sprouting was seen in bulbs infected with *P. polonicum*, which may have affected the volatile profile. The demonstrated effect of fungal pathogens on the volatile profile of onion bulbs could prove useful in the future development of monitoring systems for early detection of rot in stored onions.

1. Introduction

Onions are the second most produced vegetable crop in the world (Faostat, 2023) and an important staple in the diets of a vast majority of cultures around the world. As the onion bulb is a storage organ it is naturally well suited to storage for long time periods. However, the bulbs are susceptible to infection by a variety of fungal and bacterial pathogens and infections that start during cultivation or at harvest may sometimes become apparent only after the bulbs are already in storage. The amount of food loss caused by quality problems arising during storage differs depending on many different pre- and postharvest factors such as cultivar choice, mechanical damage, curing, pathogen infections and storage conditions (Petropoulos et al., 2017). As an example to illustrate the size of the problem, approximately 13 % of onions placed in storage in Sweden are lost by the point of sale (Franke et al., 2013), though the rate and cause of losses vary worldwide.

Due to the nature of large-scale onion storage, where bulbs are stored in large capacity bins, it is often difficult to detect pathogen-induced decay before the planned selling point. The changing headspace volatiles released by stored crops have may be key to identifying quality problems, using technology such as gas sensor arrays. Studies have shown that gas sensor arrays, also called electronic noses, have potential applications in crop storage facilities where they could aid in detecting problems such as disease or insect damage (Seesaard et al., 2022). A gas sensor array has previously been shown to successfully distinguish between healthy onions and onions infected with a fungal or bacterial pathogen in lab conditions (Labanska et al., 2022; Li et al., 2011).

One important onion pathogen that frequently causes disease in several stages of onion development, from sprouting to post-harvest bulb storage, is *Fusarium oxysporum* f.sp. *cepae*, (*F. oxysporum*) the causal agent of *Fusarium* basal plate rot. *Fusarium* basal plate rot is a widespread problem, occurring in most parts of the world where *Allium* spp. are produced, potentially causing losses of up to 30–40 % in stored bulbs (Le et al., 2021). The disease was reported by Schwartz and Mohan

* Corresponding author.
E-mail address: Isabella.Kleman@slu.se (I. Kleman).

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(2007) as having caused economically significant losses in a number of countries, from the USA to South Africa and Japan. *Fusarium oxysporum* invasion is promoted by wounds, but the fungus is also capable of penetrating healthy tissue and often invades through the roots (ibid.). The disease involves breakdown of the bulb's basal plate and eventually also the surrounding tissue. The fungal growth and breakdown of onion tissue caused by a *F. oxysporum* infection is accompanied by a change of emitted volatile organic compounds (Wang et al., 2019; Wesoly et al., 2024). A few previous studies have investigated the connection between *F. oxysporum*-infection and volatile profile change with the goal of identifying key odours that could be of use for detection (Prithiviraj et al., 2004; Vikram et al., 2005; Wang et al., 2019; Wesoly et al., 2024). Most of the studies indicate a number of sulfurous volatile organic compounds, typically released by damaged onion tissue, to be the main indicators of *Fusarium* infection. Due to differences in study design, methods for sampling and analysis and choice of onion cultivar and pathogen strains the exact list of compounds indicated as infection markers differ, though several compound overlap. Further studies under differing conditions could help consolidate a list of the compounds that are most useful as indicators for *Fusarium* basal plate rot across varying scenarios.

Another fungal pathogen causing decay in storage is *Penicillium*, with several different species implicated as causal agents for storage diseases (Duduk et al., 2017; Dugan et al., 2014; Overy and Frisvad, 2003; Schwartz and Mohan, 2007). *Penicillium* spp. often act as opportunistic wound pathogens, invading the bulbs through pre-existing damage, but may also invade healthy tissue (Schwartz and Mohan, 2007). The subject of headspace volatiles released by *Penicillium*-infected onions is rarely studied, but Vikram et al. (2005) included *P. aurantiogriseum* in their study on postharvest pathogens of onions. A number of compounds were found to be released by bulbs infected with this pathogen, but none were unique to *Penicillium* infection.

In this study we aim to further elucidate the volatile fingerprint of *Fusarium* basal plate rot and *Penicillium* rot in stored onion bulbs as the infections develop over a period of up to 10 weeks. This would increase existing knowledge and provide additional pieces of the puzzle needed to successfully develop automatic systems for detection of storage diseases through headspace volatile changes, eventually reducing food loss. We hypothesize that the two types of infection have distinct volatile profiles, with the presence or relative amounts of specific compounds marking a difference between infected and uninfected bulbs.

2. Materials and methods

2.1. Onions

Odour sampling for the experiment was carried out in spring 2022 and 2023 for *Penicillium* and *Fusarium*, respectively. All onion (*Allium cepa* L.) bulbs used for this study were obtained from the local supermarket. Bulbs were produced in Scania, Sweden, using conventional production methods. The chosen bulbs within each experiment were of an average weight of 125 g, or 8 bulbs/kg, free from visible damage and from the same producer and sales batch.

2.2. Pathogens

Penicillium polonicum CBS 222.90 (syn. *Penicillium aurantiogriseum* var. *polonicum*) was ordered as a freeze dried culture from Westerdijk FungalBio Diversity Institute (Netherlands), and grown on potato dextrose agar (DIFCO, BD, USA).

Before the start of the *Fusarium* experiment, *Fusarium oxysporum* was obtained from an onion bulb that showed visible symptoms of *Fusarium* basal plate rot. Fungal cultures were obtained by using a sterile scalpel to scrape mycelium and spores onto six potato dextrose agar (DIFCO, BD, USA) plates acidified to a pH of 3.5 using 10 % L-(+)-tartaric acid (Merck KGaA, Germany). The plates were incubated at 25 °C until sporulation occurred. Repeated cultures were started from spores, until a pure culture was obtained. DNA was extracted from the pure culture, using a ZymoBIOMICS ™ DNA Kit (Zymo Research, USA) with no deviations from the provided protocol. Using a standard PCR method, the D1/D2 domains of the 26S ribosomal RNA gene were amplified using the primers NL1, NL4, and the internally transcribed spacer region with the primers ITS1 and ITS4 (Kurtzman and Robnett, 2003; Ljunggren Joel et al., 2019; White et al., 1990). The PCR product was sent to Eurofins Genomics Europe (Germany) for Sanger sequencing. Searches for the obtained sequences were performed using the Basic local alignment search tool (BLAST) provided by the National Center for Biotechnology Information (Altschul et al., 1990) and the sequences for the strain were found to be on average 96.46 % and up to 99.46 % similar to previously sequenced strains of *F. oxysporum*. The newly isolated strain was selected for use to infect onions in this study.

2.3. Inoculation of onions

To produce spores for inoculation, PDA (DIFCO, BD, USA) plates were inoculated with *P. polonicum* or *F. oxysporum* and incubated at 25 °C for 14 days. The plates were then rinsed with sterile deionized water and gently scraped with a sterile plastic inoculation loop. The resulting suspensions were filtered through four layers of sterile cotton butter muslin to produce spore suspensions free from mycelium. The spore concentration in the suspensions was adjusted to 1×10^5 spores ml^{-1} after counting in a counting chamber. In the case of *F. oxysporum*, macroconidia and microconidia were each counted as single spores. The bulbs were then wounded to a depth of 2 cm using a 4 mm sterile injection needle, through one point at the equator of the bulb for the *Penicillium* experiment, and through the basal plate for the *Fusarium* experiment, with the control bulbs for the latter were left unwoundedTo each injury, 100 μL of spore suspension to create infected bulbs or sterile deionized water to create control bulbs, was added and allowed to soak in for 1 h before the bulbs were individually isolated in polyamide oven bags (Toppits, Cofresco Frischhalteprodukte GmbH, Germany). Eight bulbs were infected with *F. oxysporum* and six were used as control. For the *P. polonicum* experiment, four bulbs were infected with *P. polonicum* and four were injected with sterile water, for use as the control. The bags measured 25 cm by 38 cm and were filled with ambient air and sealed with a plastic bag clip 10 cm from the top of the bag.

2.4. Headspace sampling and chemical analysis

Onion bulbs remained individually bagged and stored at 20 °C throughout the duration of the experiment, with the air being replaced once weekly, after headspace sampling. Headspace volatiles were sampled once weekly from 0 to 7 weeks post infection (WPI) for *Penicillium* and from 5 to 10 WPI for *Fusarium*. Headspace was sampled statically, using solid phase microextraction (SPME) fibers coated in 50/30 μm Divinylbenzene/Carboxen/Polydimethylsiloxane (Supelco, Bellefonte, PA). Each individual bulb was assigned a specific SPME fiber repeatedly used for sampling on different occasions, with bulb A of each treatment being assigned fiber number 1, bulb B assigned fiber number 2 etc. Prior to sampling, fibres were conditioned for 5 min in a GC inlet set to 250 °C. For sampling, the clip sealing of each bag was replaced with a plastic tie leaving just enough space for the sheath covering the fiber to pass through without damaging the bag. Fibers were exposed to the onion headspace for 45 min. Samples were analysed using an Agilent 7890-5977 GC-MS equipped with a polar 60 m DB-WAX column (0.25 mm inner diameter, 0.25 μm film, USD608325H Agilent Technologies Inc., USA) where volatiles were desorbed from the fiber for 1 min at 250 °C in the splitless injection port of the GC-MS. Helium was used as carrier gas with a flow of 35 cm/s. For *Penicillium* the GC temperature was set to start at 30 °C, held for 3 min with a ramp of 8 °C/min until 225 °C which was held for 10 min. Mass spectra were recorded in scan mode in the range 12–385 m/z at 70 eV. For the *Fusarium* samples the

starting temperature was set to 40 °C. All other parameters were the same as mentioned before. Using the same methods, a blend of n-alkanes (C7-C30, Supelco, Bellefonte, PA) was injected to create reference points for calculation of Kovats retention index. Synthetic standards of 1-(methylthio)-propane, dimethyl disulfide, methyl propyl disulfide, dipropyl disulfide, 1-octen-3-ol and 6-methyl-5-hepten-2-one were injected using the same methods for confirmation of compound identity. To enable more accurate identification of compounds, several samples of each treatment type as well as all synthetic standards and the same alkane blend were run using a non-polar 60 m HP-5MS UI column (0.25 mm inner diameter, 0.25 μm film, USD637516H Agilent Technologies Inc., USA) on an Agilent 6890-5975 GC-MS. Helium was used as a carrier gas, with a flow of 34 cm/s, and the initial temperature of the GC was set to 50 °C, held for 2 min with a ramp of 8 °C/min until 250 °C which was held for 10 min. Mass spectra were recorded in scan mode in the range 29–400 m/z at 70 eV Other compounds were tentatively identified by comparing mass spectra using NIST library searches (NIST MS Search 2.4, 12th edition (2020) libraries mainlib, replib, nist_ri, w12leg, w12q, w12main, w12rep). Identities were further corroborated by comparing our calculated Kovats retention indices with those obtained by others using similar columns, where such information was available from the NIST Chemistry WebBook (https://webbook.nist.gov/chemistry/, retrieved 2024-08-08) or NIST MS Search.

Immediately after the last sampling of volatiles all bulbs were photographed to visually document the degree of infection. The bulbs infected with *F. oxysporum* and their corresponding controls were also split in half vertically and photographed.

2.5. Data analysis

Compounds that were found to occur in only one sample, in runs of clean SPME fibers or samples of empty oven bags were removed from further analysis, but were included as part of the total chromatogram area. Absolute concentrations or release rates of compounds cannot be determined using the methods employed in this study. To analyse relative quantity, percentage peak areas of target compounds were calculated as a percentage of the total area (AUC) of all detected peaks in each chromatogram, in order to reduce the influence of varying amounts of volatiles being captured due to minor differences in the sampling environment between occasions.

Principal component analysis (PCA) was carried out using the prcomp function in the stats package of R (RStudio 2024.04.2 + 764 "Chocolate Cosmos", R 4.3.3), and results were visualized using the factoextra package (Kassambara, 2016). Principal component analysis and visualisation of the time points showed strong overlap between time points for both experiments. All time points were therefore pooled for the further analysis.

Multilevel pattern analysis (multipatt) from the indicator species (indicspecies) package (Cáceres and Legendre, 2009) was used to identify indicator compounds for the different treatments.

3. Results

3.1. Fusarium

A total of 34 compounds that could be tentatively identified by mass spectra comparisons using NIST library searches were found (Fig. 1). After comparisons with previously published retention indices, the

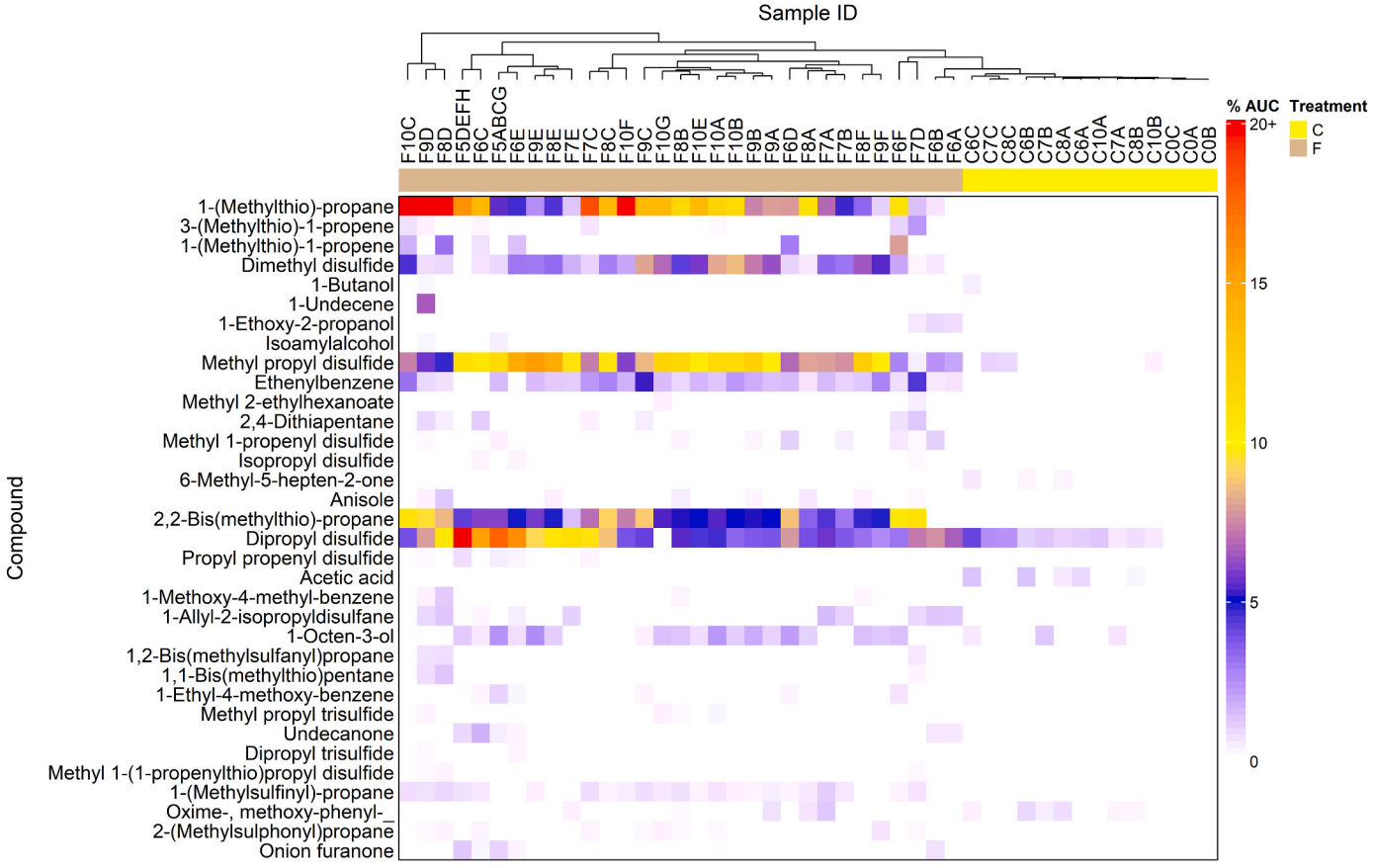

Fig. 1. Relative abundance, as percent of total area under the total ion chromatogram curve (% AUC) of volatile organic compounds found in the headspace of control (uninfected) onions and onions infected with *Fusarium oxysporum*. The x axis shows individual sampling occasions as control (C) or *Fusarium* (F), weeks post infection (0–10) and identity (A–H) of the sampled bulb.

compounds were either found to match previously published retention indices (N = 25) or had no such data available (N = 9). With all time points pooled the average total chromatogram raw data peak area was approximately 20 times higher for samples of the *Fusarium* treatment than for control samples. The multilevel pattern analysis based on the % AUC data found that 9 compounds were significant indicators for the *Fusarium* treatment (Table 1). Of those compounds, 1-(methylthio)-propane, dimethyl disulfide, 2,2-bis(methylthio)-propane, ethenylbenzene, 1-(methylsulfinyl)-propane, 2-(methylsulfonyl)propane and methyl 1-propenyl disulfide were found exclusively in the infected bulbs. The infected bulbs showed different degrees of infection (Fig. 2) and many of the control and infected bulbs had internal dry scales and displayed signs of secondary infections (Figs. 2 and 3). The biplot visualizing the principal component analysis (Fig. 4) shows the dense clustering of the control samples, diverging from the cluster of *Fusarium* samples. The effect of each of the 12 compounds found to be indicators of either *Fusarium* or control (Table 1) is also shown.

3.2. *Penicillium*

A total of 33 compounds that could be tentatively identified by mass spectra comparisons using NIST library searches and retention index comparisons were found (Fig. 5). Principal component analysis of the % AUC of the VOCs revealed separate clustering of the *Penicillium* and control treatments (Fig. 8). The separation between the clusters for the infected and non-infected bulbs was less clear for *Penicillium* than for the *Fusarium* experiment (Fig. 4). Multilevel pattern analysis revealed 5 compounds to be indicators for *Penicillium* infection (Table 2). Two compounds, 1-ethoxy-2-propanol and 2-methyl-5-pentanolide were found exclusively in infected bulbs. However, their presence in the infected bulbs was not consistent, occurring 6 and 9 times out of 23 samplings, respectively. 2,4-Dimethyl-thiophene was found in 19 of 23 infected bulb samplings and in bulb C of the water-injected control. Bulb C was the only control bulb that showed signs of fungal infection near the injection site (Fig. 7). Seven compounds were found mainly or exclusively in the water-injected control, including phenol, 6-methyl-5-hepten-2-one and several aldehydes and alcohols.With all time points pooled the average total chromatogram raw data peak area was similar for P and C, with P having approximately double the average total. The infected bulbs showed signs of infection toward the end of the sampling period in the form of mycelium and spores around the roots and sunken areas around the point of injection (Fig. 6). The infected bulbs also started sprouting as early as two weeks into the experiment, and as all infected bulbs had significant sprout and root growth and bulb shrinkage by the seventh week, the experiment was terminated after this sampling point. The water-injected bulbs did not sprout during the course of the experiment (Fig. 7), but as they also started to lose firmness the last control samples were taken at the 6 week post infection time point.

4. Discussion

In this study, we set out to further elucidate the headspace volatile profiles of onion bulbs infected with *Fusarium oxysporum* or *Penicillium polonicum.* We found that *F. oxysporum*-infection had a marked effect on the types of volatile compounds released and their relative abundance, when compared to uninfected bulbs. Infection by *P. polonicum* had a less distinct effects on the headspace volatile profiles of infected bulbs as compared to water-injected controls. Previous studies have employed several different methods in order to identify the volatile organic compounds of relevance for detecting specific diseases, including SPME followed by GC-MS (Wang et al., 2016, 2019) and direct sampling using GC–MS HAPSITE (Prithiviraj et al., 2004; Vikram et al., 2005). Prithiviraj et al. compared the volatile profiles of uninfected bulbs and bulbs infected with *Botrytis allii, Pectobacterium carotovorum* ssp. *carotovorum,* or *F. oxysporum*, and found that the tentatively identified compounds 1-oxa-4,6-diazacyclooctane-5-thione and 4-mercapto-3-(methylthio)-ç-(thio-lactone)-crotonic acid were exclusively found in onion bulbs with a *F. oxysporum* infection. Vikram et al., (2005) similarly compared the volatile profiles of bulbs infected with the same three pathogens, but also bulbs infected with *Aspergillus niger* or *Penicillium aurantiogriseum.* Their study found that tentatively identified ethyl cyclobutane was the only compound exclusively found in *F. oxysporum*-infected bulbs. We did not find these compounds in our study, nor were they reported in the papers by Wang et al. (2016, 2019) or Wesoly et al. (2024). In their 2019 study, Wang et al. used authentic reference compounds to verify the identity of the compounds found in *F. oxysporum* infected bulbs and found that 1-propanethiol, methyl propyl sulfide and ethenylbenzene (syn. styrene) were released in significant quantities starting soon after infection with a pathogenic *F. oxysporum* strain, and that ethenylbenzene was exclusively released by infected bulbs. As the released amounts of these compounds also correlated positively with the extent of fungal growth, as measured via DNA ratios, it was concluded that these compounds, among others, may be key in identifying the development of basal plate rot early. In our present study we similarly found that both ethenylbenzene and methyl propyl disulfide were indicators for *F. oxysporum*-infection. Ethenylbenzene has previously been shown to be released in relatively large quantities by *Fusarium oxysporum* growing on PDA (Beck et al., 2008). As it is also released in increased quantities by apples infected with *Penicillium expansum*, *Botryosphaeria dothidea,* and *Alternaria alternate* (Kim et al., 2018) *it is likely useful as a general indicator of fungal infection.* We can also corroborate the previously reported increased relative release of the compounds 1-(methylthio)-propane (Wang et al., 2019), dimethyl disulfide (Vikram m.fl., 2005; Wang m.fl., 2019, Wesoly et al., 2024) and 2,2-bis(methylthio)-propane (Wang et al., 2019; Wesoly et al., 2024) by

Table 1
The indicator compounds for *Fusarium oxysporum* treated onion bulbs and control bulbs, as indicated by multilevel pattern analysis from the indicator species package in R, using relative abundance, calculated as percentage of the total area under the chromatogram for each run (AUC %). Compounds were tentatively identified using a library search and mass spectra comparisons (NIST MS Search 2.4). Numbers in superscript indicate further methods used to identify the compounds, according to the following: 1 Synthetic standard injected, 2 Retention index comparison, polar column, 3 Retention index comparison, nonpolar column.

Indicators for *Fusarium*	CAS	DBWax RI	HP5 RI	Occurs in F	Occurs in C	stat	p.value
Methyl propyl disulfide[1,2]	2179-60-4	1214.9	934.6	31/31	2/13	0.992	0.005
1-(methylthio)-propane[1]	3877-15-4	n/a	n/a	30/31	0/13	0.984	0.005
Dimethyl disulfide[1,2]	624-92-0	1033	n/a	29/31	0/13	0.967	0.005
2,2-bis(methylthio)-propane[3]	6156-18-9	1357	1036.8	29/31	0/13	0.967	0.005
Ethenylbenzene[2,3]	100-42-5	1235.4	890.7	23/31	0/13	0.95	0.005
1-(methylsulfinyl)-propane	14094-08-7	1696	n/a	24/31	0/13	0.861	0.005
1-octen-3-ol[1,2,3]	3391-86-4	1427.4	978.6	19/31	3/13	0.715	0.02
2-(Methylsulfonyl)propane	4853-74-1	1948.1	n/a	11/31	0/13	0.568	0.045
Methyl 1-propenyl disulfide[2]	5905-47-5	1272.4	n/a	7/31	0/13	0.539	0.04
Indicators for control							
Acetic acid	64-19-7	1414.8	n/a	0/31	5/13	0.598	0.005
Oxime-, methoxy-phenyl-_	67160-14-9	1707.7	n/a	5/31	6/13	0.543	0.04
6-Methyl-5-hepten-2-one[2]	110-93-0	1319.8	n/a	0/31	3/13	0.463	0.025

Fig. 2. The eight sampled bulbs with *Fusarium* infections, halved and photographed immediately after the last odour samples had been taken, 10 weeks post infection. The degree of infection varied, and some bulbs (e.g. b, g and h) showed signs of additional fungi being present, in the form of dark green spores. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Fig. 3. Six control bulbs with no induced infections halved and photographed immediately after the last odour samples had been taken. Bulb a, b and c were used for headspace sampling. Spores from naturally occurring fungi can be seen on the dry roots on some of the bulbs.

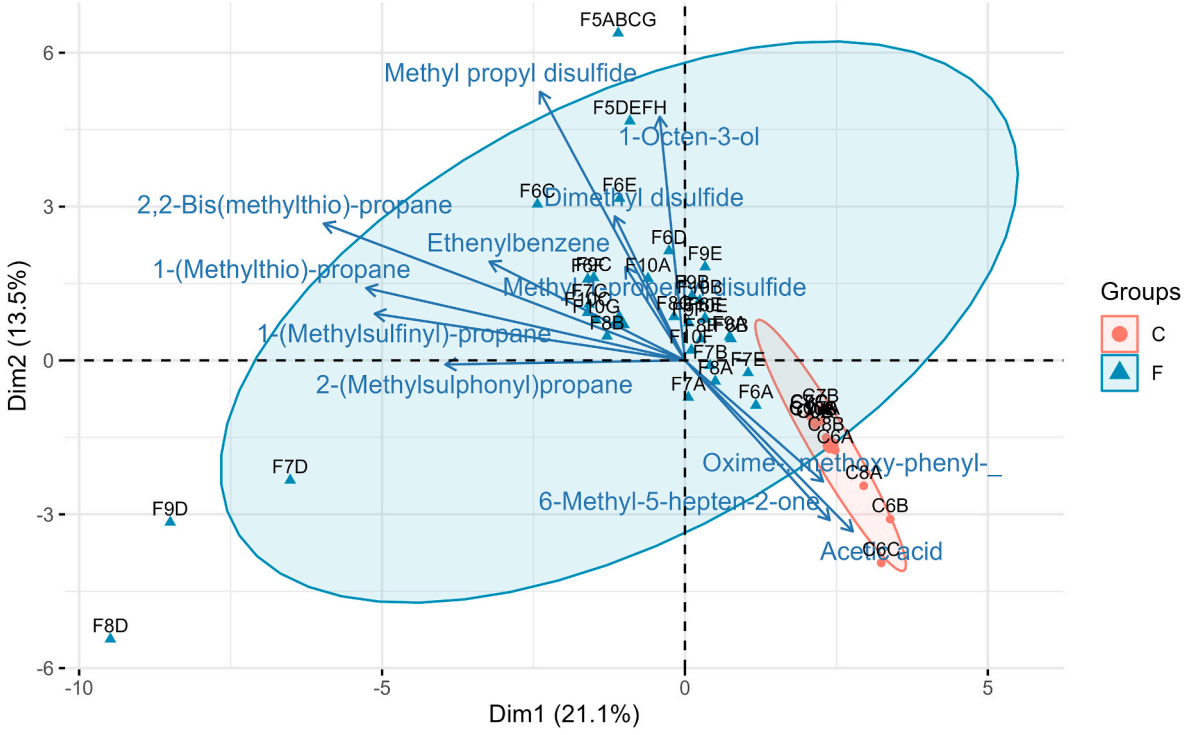

Fig. 4. Principal component analysis biplot showing the headspace volatile organic compounds marking the difference between *Fusarium oxysporum*-infected onion bulbs (F) and control bulbs (C), as indicated by a multilevel pattern analysis with a significance level of 0.05 (Table 1).

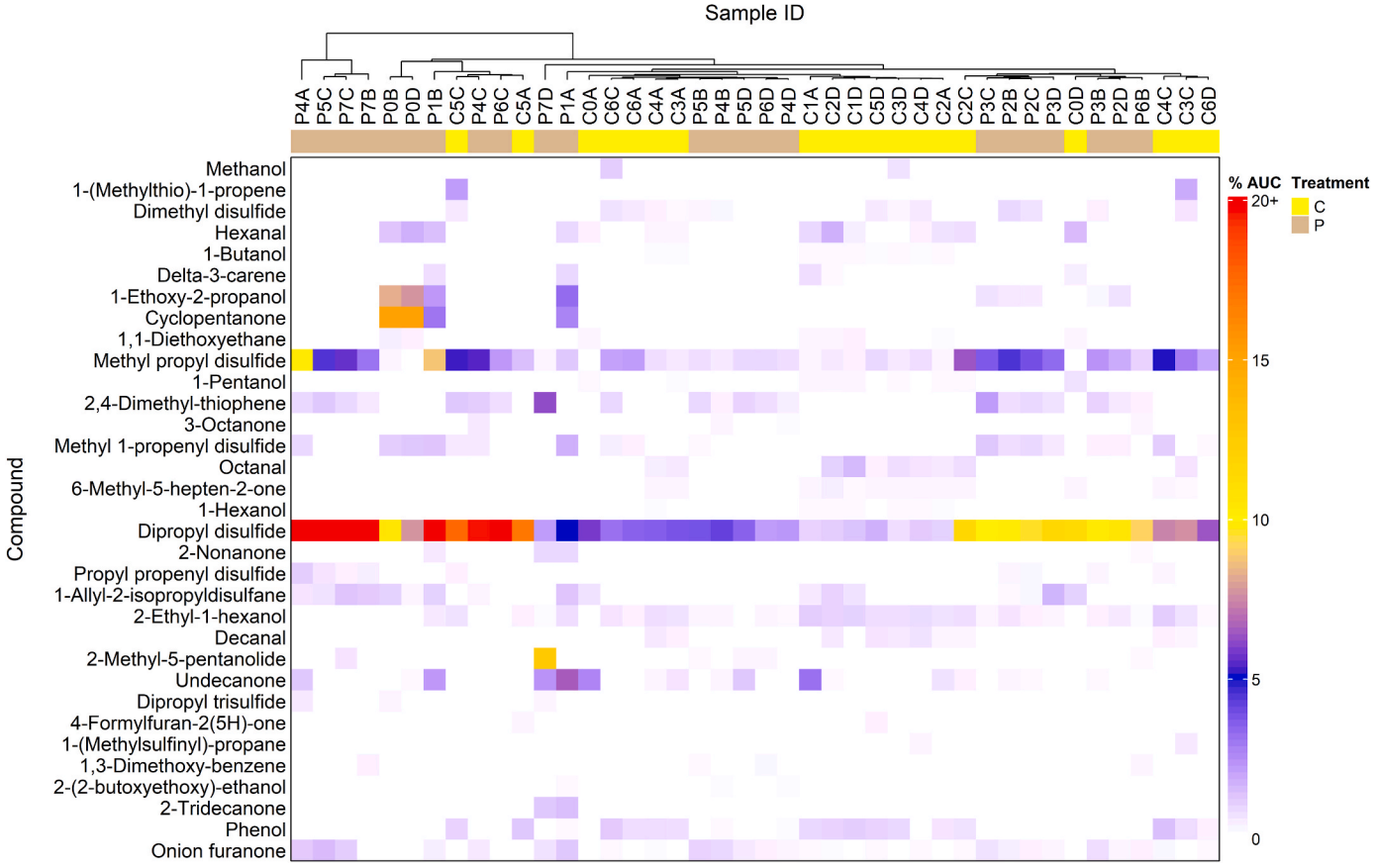

Fig. 5. Relative abundance, as percent of total area under the total ion chromatogram curve (% AUC) of volatile organic compounds found in the headspace of onions infected with *Penicillium polonicum* and control onions. The x axis shows individual sampling occasions as control (C) or *Penicillium* (P), weeks post infection (0–7) and identity (A–D) of the sampled bulb.

Table 2
The indicator compounds for *Penicillium* (P) treated onion bulbs and control (C) bulbs, as indicated by multilevel pattern analysis from the indicator species package in R, using relative abundance, calculated as percentage of the total area under the chromatogram for each run (AUC %). Compounds were tentatively identified using a library search and mass spectra comparisons (NIST MS Search 2.4).Numbers in superscript indicate further methods used to identify the compounds, according to the following: 1 Synthetic standard injected, 2 Retention index comparison, polar column, 3 Retention index comparison, nonpolar column.

Indicators for *Penicillium*	CAS	DBWax RI	HP5 RI	Occurs in P	Occurs in C	stat	p.value
2,4-Dimethyl-thiophene[2,3]	638-00-6	1241.2	910.0	19/23	2/19	0.86	0.005
2-Hexyl-5-methyl-3(2H)-furanone[2,3]	33922-66-6	2044.0	1445.9	19/23	9/19	0.849	0.005
Methyl 1-propenyl disulfide[2,3]	5905-47-5	1279.1	942.4	13/23	5/19	0.663	0.02
1-Ethoxy-2-propanol[2]	1569-02-4	1147.2	n/a	9/23	0/19	0.626	0.005
2-Methyl-5-pentanolide	n/a	1569.3	n/a	6/23	0/19	0.511	0.02
Indicators for Control							
Phenol[2]	108-95-2	1975.4	n/a	3/23	16/19	0.907	0.005
6-Methyl-5-hepten-2-one[1,2]	110-93-0	1325.8	n/a	0/23	13/19	0.827	0.005
Octanal[2]	124-13-0	1279.2	n/a	0/23	10/19	0.725	0.005
Decanal[2]	112-31-2	1492.9	n/a	0/23	10/19	0.725	0.005
1-Butanol[2]	71-36-3	1126.1	n/a	0/23	9/19	0.688	0.005
1-Pentanol[2]	71-41-0	1235.7	n/a	0/23	9/19	0.688	0.005
1-Hexanol[2]	111-27-3	1336.6	n/a	0/23	7/19	0.607	0.01

Fig. 6. The four sampled bulbs with *Penicillium* infections, photographed immediately after the last odour samples had been taken, 7 weeks post infection. Onion bulbs a-d and their respective basal plate areas are shown. Signs of infection can be seen as sunken-in areas around the site of infection and fungal growth on the dry roots. The infected bulbs also sprouted to varying degrees.

onions infected with *F. oxysporum*. Additionally, we found that 1-octen-3-ol, 1-(methylsulfinyl)-propane and 2-(methylsulfonyl)propane and methyl 1-propenyl disulfide were useful indicators of *F. oxysporum*-infection. To our knowledge, these VOCs have not yet been reported as indicators of *F. oxysporum*-infection in onion bulbs, though Li et al. (2011) found that methyl 1-propenyl disulfide was released by bulbs infected with *Burkholderia cepacia* or *Botrytis allii* but not by uninfected bulbs. As such, the increased relative release of methyl 1-propenyl disulfide may be an indicator of infection, but likely not specific to *Fusarium* infection. 1-Octen-3-ol is a well-known fungal metabolite (Assaf et al., 1997), but has not previously been reported as an indicator of pathogen infection found in the headspace of onion bulbs. Of the indicator compounds for *F. oxysporum*-infection, 1-(methylthio)-propane, dimethyl disulfide, ethenylbenzene, 2,2-bis(methylthio)-propane, 1-(methylsulfinyl)-propane and 2-(methylsulfonyl)-propane and methyl 1-propenyl disulfide were detected only in infected bulbs. However, many of these sulfur-containing compounds have been found in samples of uninfected but macerated bulbs of *Allium fistulosum* (Kuo and Ho, 1992). The release of volatile organic sulfides is a well-known consequence of cell lysis in onion bulbs (Randle and Lancaster, 2002). It seems infection raised the released amounts of many of the onion's native sulfur compounds above the detection threshold in our study. The increased release of sulfuric compounds is of interest as markers of infection for the future development of quality monitoring systems. This was shown in a study by Labanska et al. (2022), where sensors capable of detecting sulfuric compounds were particularly important in identifying *Fusarium*-infected bulbs. While some of the large molecule indicator compounds are less suited to gas sensor detection, sensors capable of detecting release of e.g. 1-octen-3-ol and dimethyl disulfide exist (Chen et al., 2020; Zhang et al., 2021), and may prove useful in storage monitoring of onions in the future.

The part of our study relating to *Penicillium*-infection showed less clear clustering between infected and noninfected bulbs in the PCA analysis (Fig. 8). The 5 compounds 2,4-dimethyl-thiophene, 2-hexyl-5-methyl-3(2H)-furanone, methyl 1-propenyl disulfide, 1-ethoxy-2-

Fig. 7. The four sampled bulbs from the control water treatment, photographed immediately after the last odour samples had been taken, 6 weeks post treatment. Onion bulbs a-d are shown. Signs of spontaneous infection can be seen around the injection site on bulb c.

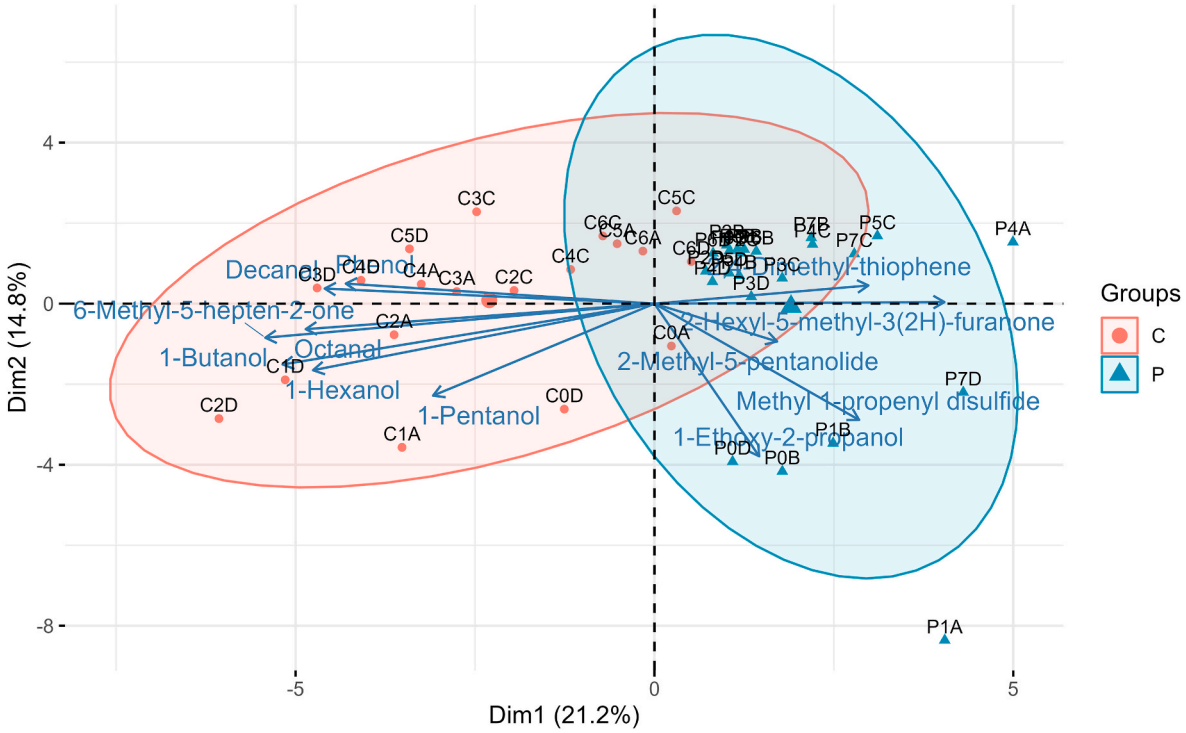

Fig. 8. Principal component analysis biplot of the headspace volatile organic compounds differing between *Penicillium polonicum*-infected onion bulbs (P) and control bulbs that were only injected with water (C), as indicated by a multilevel pattern analysis with a significance level of 0.05 (Table 2).

propanol and 2-methyl-5-pentanolide were found to be indicators of *Penicillium*-infection. However, the pattern of indicator compounds being present in most or all infected samples but none of the uninfected ones did not hold true for *P. polonicum* like it did for *F. oxysporum*. Another difference between the two experiments is that in the *Penicillium* experiment several compounds were found exclusively or in higher relative amounts in the water-injected bulbs. 2,4-Dimethyl-thiophene, 2-hexyl-5-methyl-3(2H)-furanone and methyl 1-propenyl disulfide are known to occur as natural headspace volatiles of other *Allium* species (Kuo and Ho, 1992), but the relative abundance seemed to increase in our study, making these compounds relevant as indicators of infection. It is possible that the chosen strain of *P. polonicum* has a low pathogenicity

or that *Penicillium* infection has less of an effect on headspace VOC profile than other types of infection. The latter seemed to be the case also in the study by Vikram et al. (2005), who found only small differences between wounded bulbs and bulbs infected with *Penicillium*, with tentatively identified 2-azabicyclo[3.2.0]hept-6-ene and α-2,5,7-octatrienyl-N-propylbenzenemethanamine being the only compounds that differentiated *Penicillium*-infection from wounded bulbs. Neither of these compounds were detected in our samples. The small differences between infected and control bulbs in that study indicates that *Penicillium*-infection in onion bulbs does not tend to greatly modify the volatile fingerprint of the bulbs' headspace, at least not within the first six days after infection. In our study, we sampled the *Penicillium*-infected bulbs weekly until seven weeks post infection, but found that there was still some overlap between infected and water-injected bulbs (Fig. 8). One indicator compound that was found in higher relative abundance in the infected bulbs was 2-hexyl-5-methyl-3(2H)-furanone, which is a naturally occurring onion compound, also known as onion furanone. Onion furanone was found by Li et al. (2011) to be released in increased quantities by onions infected with *Botrytis allii* and *Burkholderia cepacia* when compared to control bulbs, but it was not reported by Vikram et al. in any of their samples. In this study, we also found onion furanone in some, but not all, of the *F. oxysporum*-infected bulbs, but not in the unwounded control. It is likely that the increased release of this compound is connected to infection or injury, but it is not a marker exclusive to *Penicillium*-infection. The effect of the sprouting seen in the *Penicillium*-infected bulbs on the VOC profile is unclear, and may be of interest for future studies. Whether the increased rate of sprouting seen in the infected bulbs was an effect of the infection is another question that could be further explored.

The internal dry scales seen in bulbs of both the *F. oxysporum* treatment (Fig. 2) and the corresponding control (Fig. 3) is a quality problem occasionally seen in onion bulbs. The cause is not fully known, but is thought to be related to heat stress during bulb formation and premature leaf dieback (du Toit et al., 2016). Internal dry scales are also thought to be associated with pathogens such as *Fusarium proliferatum* or bacterial rot agents (ibid.). The region in which the bulbs used for this study were grown had a relatively hot and dry summer in 2022, which may have contributed to the quality problems seen. It was also apparent that the introduced *F. oxysporum* was not the only fungus present in neither the treated nor control bulbs, as several displayed dark green fungal spores externally or among the internal dry scales, where such scales were present. As all bulbs carry with them their own individual microbiota and cannot be sterilized, the result of introducing a pathogenic organism will differ, as becomes obvious when observing the eight replicate bulbs infected with *F. oxysporum* in this study (Fig. 2). As a result of the differing degrees of infection, the volatile profiles differed somewhat between the advanced decay of bulb FD and the less severely infected bulbs in the *Fusarium* treatment (Fig. 4). Nonetheless, all artificially *Fusarium*-infected bulbs had several headspace VOCs in common that differentiated them from the control. The study has limitations in that the number of replicates was low and that sampling was carried out in lab conditions rather than in commercial storage. Authentic reference compounds were used only for certain of the indicator compounds, while others were identified based on a combination of mass spectral similarity and retention indices, where available, from polar and nonpolar GC columns. The findings are in line with previously published findings from similar studies, with the addition of a few new possible indicator compounds, suggesting the results are plausible.

5. Conclusion

We found that methyl propyl disulfide, 1-(methylthio)-propane, dimethyl disulfide, 2,2-bis(methylthio)-propane, ethenylbenzene, 1-(methylsulfinyl)-propane, 1-octen-3-ol, 2-(methylsulfonyl)propane and methyl 1-propenyl disulfide were compounds of interest as markers of *F. oxysporum*-infection in onions. 1-Octen-3-ol and methyl propyl disulfide were detected only rarely in control bulbs, and all other indicator compounds were detected exclusively in *F. oxysporum*-infected bulbs. Characteristic volatiles released by *P. polonicum*-infected bulbs were less obvious, but certain compounds including 2,4-dimethyl-thiophene, onion furanone and methyl 1-propenyl disulfide were more abundant in the infected bulbs. As methyl 1-propenyl disulfide was found to be an indicator for both *Fusarium* and *Penicillium* infection, it could prove useful as a more general indicator of infection. Future steps involving the development, verification and deployment of gas sensor arrays, targeting the smaller molecular size indicator compounds, could put this information to use in preventing storage losses of onions.

CRediT authorship contribution statement

Isabella Kleman: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Anna Karin Rosberg:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Oleksiy Guzhva:** Writing – review & editing, Visualization, Supervision, Data curation, Conceptualization. **Maria E. Karlsson:** Writing – review & editing, Methodology, Formal analysis. **Paul G. Becher:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization. **Lars Mogren:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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